

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problems Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|---|
| (51) International Patent Classification ⁶ : C12Q 1/68, C12P 19/34, C07H 21/02, 21/04 | A1 | (11) International Publication Number: WO 97/41263 (43) International Publication Date: 5 November 1997 (06.11.97) |
| (21) International Application Number: PCT/US97/07676 (22) International Filing Date: 28 April 1997 (28.04.97) (30) Priority Data: 08/640,389 29 April 1996 (29.04.96) US (71) Applicant: PROGENITOR, INC. [US/US]; 1507 Chambers Road, Columbus, OH 43212 (US). (72) Inventors: SNODGRASS, Ralph, H.; 650 Retreat Lane, Powell, OH 43065 (US). CIOFFI, Joseph; 1180 Bayboro Drive, New Albany, OH 43054 (US). ZUPANCIC, Thomas, J.; 501 Park Boulevard, Worthington, OH 43085 (US). SHAFER, Alan, W.; 256 Lakeview Drive, Lancaster, OH 43130 (US). (74) Agents: POISSANT, Brian, M. et al.; Pennie & Edmonds L.L.P., 1155 Avenue of The Americas, New York, NY 10036 (US). | | (81) Designated States: AU, CA, IL, JP, KR, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> |
| (54) Title: DETECTION OF THE LEPTIN RECEPTOR IN REPRODUCTIVE ORGANS AND METHODS FOR REGULATING REPRODUCTIVE BIOLOGY (57) Abstract <p>The present invention relates to variant forms of the receptor for the <i>obese</i> gene product. In particular, the invention relates to methods of detecting receptor variants in the reproductive organs for the diagnosis of the cause of infertility. In addition, it relates to methods of inhibiting or down-regulating expression of defective variants in cells to augment their responsiveness to regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system to improve fertility.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | KR | Republic of Korea | PL | Poland | | |
| CN | China | | | PT | Portugal | | |
| CU | Cuba | KZ | Kazakhstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

DETECTION OF THE LEPTIN RECEPTOR
IN REPRODUCTIVE ORGANS AND METHODS
FOR REGULATING REPRODUCTIVE BIOLOGY

1. INTRODUCTION

5 The present invention relates to variant forms of the
receptor for the obese gene product. In particular, the
invention relates to methods of detecting receptor variants
in the reproductive organs for the diagnosis of the cause of
infertility. In addition, it relates to methods of
10 inhibiting or down-regulating expression of defective
variants in cells to augment their responsiveness to
regulation by leptin as well as methods of using compounds to
directly activate signal transduction pathways associated
with this ligand-receptor system to improve fertility.
15

2. BACKGROUND OF THE INVENTION

Infertility is a major clinical problem in Western
societies. A number of contributing factors have been
identified for infertility, which include metabolic diseases
20 in a male that result in insufficient sperm production and
the inability of a female's ovaries to produce or release
ova. In addition, pituitary disorders may cause infertility
in both sexes because the gonads are responsive to regulation
by pituitary hormones such as follicle-stimulating hormone
25 and luteinizing hormone. However, clinical conditions exist
in which an infertile female is still capable of ovulating.
Therefore, the ovary may respond to additional signals that
are not yet identified.

30 Zhang et al. (1994, *Nature* 372:425-432) describe the
cloning and sequencing of the mouse *ob* gene and its human
homolog. In an effort to understand the physiologic function
of the *ob* gene, several independent research groups produced
recombinant *ob* gene product in bacteria for *in vivo* testing
(Pelleymounter et al., 1995, *Science* 269:540-543; Halaas et
35 al., 1995, *Science* 269:543-546; Campfield et al., 1995,
Science 269:546-549). When the Ob protein (also known as
leptin) was injected into grossly obese mice, which possessed

two mutant copies of the *ob* gene, the mice exhibited a reduced appetite and began to lose weight. Similarly, when normal mice received leptin, they also ate less than the untreated controls. Interestingly, when leptin was
5 administered to *ob/ob* female mice which were always infertile, fertility was restored in these animals (Chenab et al., 1996, *Nature Genetics* 12:318-320).

Recently, a leptin fusion protein was generated and used to screen for the leptin receptor (also known as OB-R) in a
10 cDNA expression library prepared from mouse choroid plexus, a tissue that lines brain cavities termed ventricles (Tartaglia, 1995, *Cell* 83:1263-1271). This approach led to the cloning of one form of the OB-R coding sequence, which reveals a single membrane-spanning receptor, sharing
15 structural similarities with several Class I cytokine receptors, such as the gp130 signal-transducing component of the interleukin-6 receptor (Taga et al., 1989, *Cell* 58:573-581), the granulocyte-colony stimulating factor receptor (Fukunaga et al., 1990, *Cell* 61:341-350), and the leukemia
20 inhibitory factor receptor (Gearing et al., 1991, *EMBO J.* 10:2839-2848). Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) demonstrate that OB-R mRNA is expressed in several tissues, including lung, kidney, total brain, choroid plexus and hypothalamus.

25 The reported mouse OB-R protein contains a relatively short intracellular cytoplasmic domain as compared with other Class I cytokine receptors. Subsequently, when cDNA encoding its human homolog was isolated from a human infant brain library, the predicted human protein sequence contains a much
30 longer intracellular domain. In view of this finding, it was speculated that different forms of the receptor might exist (Barinaga, 1996, *Science* 271:29). However, prior to the present invention, there was no report on how variant forms of the OB-R in humans would relate to infertility.

35

3. SUMMARY OF THE INVENTION

The present invention relates to variant forms of the human OB-R. In particular, it relates to the detection of these receptor variants in reproductive organs such as the ovary and the prostate gland for diagnosis of the cause of infertility, and methods for treating infertility by targeting these variant receptors.

The invention is based, in part, upon the Applicants' discovery of human cDNA clones encoding three variant forms of the OB-R. These receptors differ structurally from a reported OB-R with only three amino acid substitutions in the extracellular domain, but extensive diversity is observed in their intracellular cytoplasmic domains at the 3' end. The cytoplasmic domains of the variants of the invention are both shorter and distinct in nucleotide sequence from the corresponding domain of the published form of OB-R (Tartaglia et al., 1995 Cell 83:1263). In addition, the cytoplasmic domain of one such variant is highly homologous to a human retrotransposon sequence. The OB-R variants described herein represent incomplete receptors which may be incompetent or partially competent in transducing signals upon ligand binding. Expression of the different forms of the receptor have been detected in prostate and ovary. Furthermore, leptin activity has been shown to be naturally present in ovarian follicular fluids. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the detection of the receptor variants in reproductive organs for the diagnosis of infertility, methods to inhibit and/or down-regulate the expression of these receptor variants, gene therapy to replace the receptor variants in homozygous individuals, and direct activation of downstream signal transduction pathways in cells expressing the defective receptor variants for improving fertility.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1E. Nucleotide sequence and deduced amino acid sequence of Form 1 the human OB-R variant.

The amino acid sequence diverges from the human OB-R reported by Tartaglia et al. (1995, Cell 83:1263-1271) at nucleotide residue #349, #422, #764 and from residue #2770 and beyond.

5

Figure 2.

Nucleotide sequence comparison between human OB-R variant Form 1, Form 2 and Form 3 at the 3' end.

10 Figure 3A-3C.

Amino acid sequence comparison between OB-R variant Forms 1 (HuB1.219-1), 2 (HuB1.219-2), 3 (HuB1.219-3), human OB-R (HuOBR) published by Tartaglia et al., 1995, Cell 83:1263; and murine OB-R (MuOBR).

15

Figure 4.

Proliferation of BaF3 cells and cells transfected with chimeric OB-R in the presence of follicular fluids. ■ = transfected cell line; □ = BaF3 parent cell line.

20

Figure 5.

Proliferation of transfected BaF3 cell line in the presence of follicular fluids is inhibited by soluble OB-R, indicating that leptin is the active growth-inducing substance in the fluids. ■ = transfected cell line; □ = BaF3 parent cell line.

25

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE OB-R VARIANTS

30

The present invention relates to nucleic acid and amino acid sequences of OB-R variants. In a specific embodiment by way of example in Section 6, *infra*, three variants were cloned and characterized. Amino acid sequence comparison of these OB-R variants with a published human OB-R sequence

35 (Tartaglia et al., 1995, Cell 83:1263-1271) reveals three amino acid differences in their extracellular domains and extensive diversity in their intracellular cytoplasmic

domains. More specifically, Figure 1A-1E shows that in the variants, nucleotide residues #349-351 encode alanine, nucleotide residues #421-423 encode arginine and nucleotide residues #763-765 encode arginine. Additionally, the 5 variants diverge both in length and sequence composition from the human OB-R sequence published by Tartaglia et al. from nucleotide residue #2770 and beyond (Figure 2). In this regard, the intracellular domain of Form 1 (Figure 1A-1E) of the variants is highly homologous to a retrotransposon 10 sequence (Ono et al., 1987, Nucl. Acid. Res. 15:8725-8737). Such variants represent functionally defective forms of human OB-R in signal transduction upon leptin binding.

In order to clone additional variant forms of the molecule, labeled DNA probes made from nucleic acid fragments 15 corresponding to any portion of the cDNA disclosed herein may be used to screen a cDNA library prepared from human ovary, human prostate, human fetal liver, human lung, human kidney, human choroid plexus and human hypothalamus. More specifically, oligonucleotides corresponding to either the 5' 20 or 3' terminus of the cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 25 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in 30 neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon 35 sperm DNA at 0.5 mg/ml for 6 hours at 60°C. The radiolabelled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution

containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M EDTA) twice for 5 minutes each at room temperature, then in 5 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is aligned with the filters to select a positive plaque. If a 10 single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCl, pH 7.5, 0.01% gelatin. The phage may then be replated and rescreened to obtain single, well 15 isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

One method for identifying all 3' isoforms is to PCR 20 amplify the 3' ends of the variant cDNA from a variety of tissues including but not limiting to, choroid plexus, hypothalamus, fetal liver, bone marrow, ovary, or prostate. To obtain the 3' end of the cDNA, an oligo-dT primer is used to synthesize the cDNA first strand. OB-R specific primers 25 from the conserved region of the gene (e.g. up stream of nucleotide 2770) and oligo-dT are then used to amplify the 3' end. The PCR fragments are cloned and sequenced by standard techniques. Once obtained, these sequences may be translated into amino acid sequence and examined for certain landmarks 30 such as continuous open reading frame, regulatory regions that associate with tyrosine kinase activation, and finally overall structural similarity to known OB-R variants. These 3' variants may represent additional signal transduction defective forms of OB-R.

35

5.2. EXPRESSION OF THE OB-R VARIANTS

In accordance with the invention, the OB-R variant polynucleotide sequence which encodes a protein, peptide fragments, fusion proteins or functional equivalents thereof, 5 may be used to generate recombinant DNA molecules that direct the expression of the protein, peptide fragments, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a 10 part of such polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a 15 functionally equivalent amino acid sequence, may be used in the practice of the invention for the expression of the OB-R variant. Such DNA sequences include those which are capable of hybridizing to the OB-R variant sequence under stringent conditions, particularly at its 3' end. The phrase 20 "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, 25 for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at PH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, 30 sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or 35 substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions,

additions or substitutions of amino acid residues within the OB-R variant sequence, which result in a silent change thus producing a functionally equivalent protein. Such amino acid substitutions may be made on the basis of similarity in
5 polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged
10 polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

15 The DNA sequence of the invention may be engineered in order to alter the OB-R variant coding sequence for a variety of ends, including but not limited to, alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which
20 are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. In addition, the intracellular domain may also be altered and replaced by a different domain, such as the OB-R intracellular domain by
25 Tartaglia et al.

In another embodiment of the invention, the OB-R variant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or stimulators of receptor activity,
30 it may be useful to encode a chimeric protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the OB-R variant sequence and the heterologous protein sequence, so that the
35 variant may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of the OB-R variant could be synthesized in whole or

in part, using chemical methods well known in the art. (See, for example, Caruthers et al., 1980, *Nuc. Acids Res. Symp. Ser.* 7:215-233; Crea and Horn, 1980, *Nuc. Acids Res.* 9(10):2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters* 21:719; and Chow and Kempe, 1981, *Nuc. Acids Res.* 9(12):2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize OB-R variant amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, *Proteins Structures And Molecular Principles*, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

In order to express the OB-R variant in host cells, the nucleotide sequence coding for the variant, or a functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. The expressed gene products as well as host cells or cell lines transfected or transformed with recombinant OB-R variant expression vectors can be used for a variety of purposes. For example, host cells expressing the OB-R variant may be used to verify the ability of this molecule to bind leptin in a binding assay with radiolabeled, enzyme-conjugated or fluorescent dye-conjugated leptin. At the same time, the ability of the molecule to transduce an activation signal in host cells upon binding to leptin may be tested by assaying proliferation or phosphorylation pattern of kinases in the cells. In addition, genetically-engineered host cells can be used to screen for and select agonist and antagonist compounds, including any inhibitors that would interfere with binding of leptin to the extracellular or intracellular domain of the OB-R variant. In that connection, such host

cells may be used to screen for and select small molecules
i.e., peptides, nucleic acids and synthetic compounds that
can supplement the incomplete signal transduced by the OB-R
variant following leptin binding. Such small molecules may
5 also affect receptor isoform pairing, thereby modifying the
ability of OB-R to respond to leptin. Other uses, include,
but are not limited to generating antibodies (i.e.,
monoclonal or polyclonal) that competitively inhibit activity
of an OB-R variant, neutralize its activity, or even enhances
10 it activity. Antibodies may be used in detecting and
quantifying expression of OB-R levels in cells and tissues.

5.3. USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be used for
15 diagnostic and/or therapeutic purposes. For diagnostic
purposes, an OB-R variant polynucleotide may be used to
detect gene expression or aberrant gene expression in
infertile individuals as well as in normal individuals to
identify predisposition for infertility. Included in the
20 scope of the invention are oligonucleotide sequences, that
include antisense RNA and DNA molecules, ribozymes and
triplex DNA, that function to inhibit translation of OB-R
variant.

25 5.3.1. DIAGNOSTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may have a number of uses
for the diagnosis of the possible causes underlying
infertility, resulting from expression of a defective
receptor variant. For example, the OB-R variant cytoplasmic
30 domain DNA sequence may be used in hybridization assays of
biopsy or autopsy materials obtained from ovary or prostate
to diagnose OB-R variant expression; e.g., Southern or
Northern analysis, including in situ hybridization assays as
well as PCR. Such techniques are well known in the art, and
35 are in fact the basis of many commercially available
diagnostic kits. For PCR detection, primers may be designed
from a conserved region of the coding sequence and within the

3' region of OB-R variant. The tissues suitable for such analysis include but are not limited to, prostate, ovary, and testes, ova, sperm (semen), and cells in the ovarian follicular fluids.

5

5.3.2. THERAPEUTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be useful in the treatment of infertile conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not respond to leptin normally due to expression of a defective OB-R variant. In some instances, the polynucleotide encoding a functional OB-R is intended to replace or act in the place of the functionally defective OB-R variant gene. Alternatively, abnormal conditions characterized by expression of two copies of the OB-R variant can be treated using the gene therapy techniques described below.

Non-responsiveness to normal levels of leptin may contribute to infertility. This may result from a functionally defective receptor that does not transduce competent signals upon ligand binding. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express signalling competent forms of OB-R which may be used to augment the non-responsiveness of the naturally occurring OB-R variant. A signalling competent form may be, for example, a protein with the same extracellular domain and transmembrane region, but containing all or part of its normal signal transduction domain, such as that described by Tartaglia et al. (1995, Cell 83:1263-1271). Thus, recombinant gene therapy vectors may be used therapeutically for treatment of infertility resulting from expression or activity of the OB-R variant. Accordingly, the invention provides a method of augmenting signal transduction by an endogenous OB-R variant in a cell comprising delivering a DNA molecule encoding a signalling competent form of the OB-R to the cell so that the signalling competent protein is produced

DES

in the cell and competes with the endogenous defective OB-R variant for access to molecules in the signalling pathway which does not activate or are not activated by the endogenous natural defective receptor. Additionally, since
5 dimerization of a functional receptor with a defective variant may occur in cells of heterozygous individuals, small molecules may be used to inhibit such pairing, thereby increasing the number of functional dimeric receptors for proper signalling in response to leptin.

10 Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant functional OB-R into the targeted cell population. Methods which are well known to those skilled in
15 the art can be used to construct recombinant viral vectors containing an OB-R polynucleotide sequence. See, for example, the techniques described in Sambrook et al., 1989, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, *Current Protocols*
20 *in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant OB-R molecules can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences including anti-sense RNA and
25 DNA molecules and ribozymes that function to inhibit the translation of the OB-R variant mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to
30 antisense DNA, oligodeoxyribonucleotides derived from the OB-R variant nucleotide sequence at nucleotide #2771 and beyond, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of
35 ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the

invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of OB-R variant RNA sequences.

Specific ribozyme cleavage sites within any potential
5 RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the
10 cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary
15 oligonucleotides, using ribonuclease protection assays.

Oligodeoxyribonucleotides can form sequence-specific triple helices by hydrogen bonding to specific complementary sequences in duplexed DNA. Interest in triple helices has focused on the potential biological and therapeutic
20 applications of these structures. Formation of specific triple helices may selectively inhibit the replication and/or gene expression of targeted genes by prohibiting the specific binding of functional trans-acting factors.

Oligonucleotides to be used in triplex helix formation
25 should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of
30 a duplex. Oligonucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich oligonucleotides provide base complementarity to a purine-rich region of a single strand of the duplex in a
35 parallel orientation to that strand. In addition, oligonucleotides may be chosen that are purine-rich, for example, containing a stretch of G residues. These

oligonucleotides will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" oligonucleotide. Switchback oligonucleotides are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

35

5.4. ACTIVATION OF TYROSINE KINASE PATHWAYS IN INFERTILITY

Many known class I cytokine receptors initiate cell signaling via Janus kinases (JAKs) (Ihle, 1995, *Nature* 377:591-594; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al, 1994, *Cell* 76:253-62; Ziemiecki et al, 1994, *Trends Cell Biol.* 4:207-212). JAK1-3 have been shown to bind to conserved sequences termed box1 and box2 (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53). Ligand binding induces a homo- or hetero-dimerization of receptor chains which activates, by phosphorylation, the JAKs. The activated JAKs, in turn, phosphorylate members of the STAT family (Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., *Blood* 86:1243-54; Darnell et al., 1994, *Science* 264:1415-21; Zhong et al, 1994, *Proc. Natl. Acad. Sci. USA* 91:4806-10; Hou et al., 1994, *Science* 265:1701-6). These phosphorylated STATs ultimately translocate to the nucleus, form transcription complexes, and regulate gene expression. Both box1 and box2 are required for complete signaling in certain systems. (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53).

The OB-R variants disclosed herein have a typical box1 (from nucleotide #2707-2730) that contains the critical xWxxxPxP amino acid sequence, but they do not contain an obvious box2 nor further downstream sequences that are important for normal receptor activation. Therefore, it is possible to use compounds that activate JAKs to directly activate these pathways for improving fertility without triggering the OB-R:

6. EXAMPLE: MOLECULAR CLONING OF OB-R VARIANTS

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (designated as Hu-B1.219 #4, #33, #34, #1, #3, #8, #36, #55, #57, #60 and #62) contained overlapping sequences,

which were then compiled into a contiguous nucleotide sequence referred to as Hu-B1.219. When the deduced amino acid sequence of one such sequence (Figure 1A-1E) was compared with the sequence of a recently published human OB-R (Tartaglia et al., 1995, Cell 83:1263), they were shown to be nearly identical in the extracellular domains with the exception of three amino acids, whereas there existed extensive diversity in their intracellular cytoplasmic domains at the 3' end. This sequence encodes an OB-R variant herein referred to as Form 1. In addition, two other variants were identified, and they both differ from the human OB-R published by Tartaglia et al (1995, Cell 83:1263) in their 3' ends (Figure 2). These two additional variants are referred to as Forms 2 and 3 of OB-R. The predicted protein sequences of the variants (Figure 3A-3C) contain two FN III domains, each containing a "WS box", which are characteristic of genes of the Class I cytokine receptor family.

When various human tissue RNA were probed with a fragment containing a sequence commonly shared by the OB-R variants by Northern blot analysis, expression was detected in heart, placenta, lung, liver, muscle, pancreas, prostate, ovary, small intestine and brain (Table I).

Based on the sequence presented in Figure 1A-1F, the translation initiation site appears at position #97. The sequences of Forms 1, 2 and 3 encode an open reading frame up to and including nucleotide #2970, #2814 and #2784 (Figure 2), respectively. It is believed that the sequence between nucleotides #2629 and #2682 (Figure 1A-1E) encodes a transmembrane domain. The complete sequences of Forms 1, 2 and 3 encode proteins of 958, 906 and 896 amino acids, respectively.

TABLE I

SUMMARY OF NORTHERN BLOT ANALYSIS OF
OB-R EXPRESSION IN HUMAN TISSUES AND CELL LINES

5

| Developmental Stage | Tissue Type | Expression |
|---------------------|------------------|------------|
| 10 fetal | brain | - |
| | lung | +++ |
| | liver | +++++ |
| | kidney | + |
| 15 adult | heart | ++ |
| | brain | +/- |
| | placenta | + |
| | lung | + |
| | liver | +++ |
| | skeletal muscle | + |
| | kidney | +/- |
| | pancreas | + |
| | spleen | +/- |
| | thymus | +/- |
| | prostate | ++ |
| | testis | +/- |
| | ovary | +++ |
| | small intestine | ++ |
| | colon | - |
| 25 | peripheral blood | - |
| | leukocytes | - |
| 30 | | |

35

| Developmental Stage | Tissue Type | Expression |
|---------------------|-------------|------------|
| 5 | cancer | |
| | HL-60 | - |
| | HeLa | - |
| | K-562 | +++ |
| | MOLT-4 | - |
| 10 | Raji | - |
| | SW480 | - |
| | A549 | + |
| | G361 | - |

The sequences of the three OB-R variants are identical to the sequence of human OB-R reported by Tartaglia (1995, Cell 83:1263-1271) in the transmembrane region and a portion of the intracellular domain up to and including nucleotide #2769 (Figure 2), then they diverge at nucleotide #2770 and beyond. In addition, the products of these cDNA are substantially shorter in their intracellular domain than the human OB-R published by Tartaglia et al. These isoforms of OB-R may derive from a common precursor mRNA by an alternative splicing mechanism. The sequence in this region is consistent with well known splice junctions. It is noteworthy that the DNA sequence of Form 1 (Figure 1A-1E) of the OB-R variant from nucleotide #2768 to the end is 98% identical to a human retrotransposon sequence that is thought to be derived from a human endogenous retroviral DNA sequence (Singer, 1982, Cell 28:433; Weiner et al., 1986, Ann. Rev. Biochem. 55:631; Lower et al., 1993, Proc. Natl. Acad. Sci. USA 90:4480; Ono et al., 1987, Nucl. Acid. Res. 15:8725-8735).

7. EXAMPLE: EXPRESSION OF OB-R IN SEX HORMONE
PRODUCING CELLS IN THE OVARY AND
DETECTION OF LEPTIN IN
OVARIAN FOLLICULAR FLUIDS

7.1. MATERIALS AND METHODS

5

7.1.1. REVERSE TRANSCRIPTION/POLYMERASE CHAIN
REACTION (RT/PCR)

Total RNA was isolated using standard laboratory
procedures (Sambrook et al., 1989, Molecular Cloning, A
10 Laboratory Manual, Cold Spring Harbor Laboratory, NY).
Approximately 1 µg of total RNA was reverse transcribed and
the cDNA was amplified by PCR (Perkin Elmer, Norwalk, CT).
The PCR amplification conditions were: 94°C for 30 sec, 60°C
for 30 sec, 72°C for 30 sec for a total of 40 cycles. The
15 amplified products were resolved by agarose gel
electrophoresis and visualized by ethidium bromide staining.
The OB-R amplimers were GGTTTGCATATGGAAGTC (upper) and
CCTGAACCATCCAGTCTCT (lower). The Form 1 specific amplimers
were GACTCATTGTGCAGTGTTTCAG (upper) and TAGTGGAGGGAGGGTCAGCAG
20 (lower).

7.2. RESULTS

Table I in Section 6, supra, shows that the OB-R is
expressed in reproductive organs such as ovary and prostate
25 gland. In order to determine the specific cell types in the
ovary that expressed the receptor, primary granulosa and
cumulus cell cultures were established from the ovaries and
assayed for OB-R expression by RT/PCR. In Table II, the
cells that produced sex hormones in the ovary, i.e. granulosa
30 and cumulus cells, expressed the different forms of OB-R.

35

TABLE II

OB-R EXPRESSION IN GRANULOSA AND
CUMULUS CELLS BY RT/PCR

| | | | | |
|----|--|--------|--------|-------|
| 5 | Cell Types | Form 1 | Form 3 | OB-R* |
| | Granulosa cells derived from ovarian follicles | + | + | +/- |
| | Granulosa cells derived from ovarian follicles | - | + | + |
| 10 | Cumulus cells derived from oocytes | + | + | + |

* OB-R refers to the published sequence by Tartaglia (1995, Cell 83:1263-1271).

15 Additionally, ovarian follicular fluids were obtained from several patients and assayed for the presence of leptin. The detection assay utilized an interleukin-3-dependent cell line, BaF3, that had been transfected with a chimeric receptor construct containing the extracellular domain of murine OB-R ligated to the transmembrane and cytoplasmic domains of the thrombopoietin receptor. Both the BaF3 parental cell line and the transfected cell line responded to IL-3, whereas only the transfected cells responded to leptin (Figure 4).

25 When the cells were incubated with follicular fluids, the transfected cell line was induced to proliferate as compared with the parental cell line as a control (Figure 4). The cell growth-stimulating activity in the fluids was shown to be leptin since the activity was specifically inhibited by the addition of soluble murine OB-R (Figure 5). Therefore, 30 leptin is present in the follicular fluids, and it stimulates OB-R-expressing cells in the ovary to proliferate.

8. DEPOSIT OF MICROORGANISMS

35 The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

| <u>Strain Designation</u> | <u>Accession No.</u> |
|---------------------------|----------------------|
|---------------------------|----------------------|

| | |
|-----------------|-------|
| HuB1.219, #1 | 75885 |
| HuB1.219, #4 | 75886 |
| HuB1.219, #8 | 75887 |
| HuB1.219, #33 | 75888 |
| 5 HuB1.219, #34 | 75889 |
| HuB1.219, #36 | 75890 |
| HuB1.219, #55 | 75971 |
| HuB1.219, #60 | 75973 |
| HuB1.219, #3 | 75970 |
| HuB1.219, #57 | 75972 |
| HuB1.219, #62 | 75974 |

10 The present invention is not to be limited in scope by
the exemplified embodiments, which are intended as
illustrations of individual aspects of the invention.
Indeed, various modifications for the invention in addition
to those shown and described herein will become apparent to
15 those skilled in the art from the foregoing description and
accompanying drawings. Such modifications are intended to
fall within the scope of the appended claims.

20 All publications cited herein are incorporated by
reference in their entirety.

25

30

35

International Application No: PCT/

| MICROORGANISMS | |
|--|--|
| Optional Sheet in connection with the microorganism referred to on page <u>36</u> , lines <u>25-37</u> of the description | |
| A. IDENTIFICATION OF DEPOSIT * | |
| Further deposits are identified on an additional sheet * | |
| Name of depositary institution * | |
| American Type Culture Collection | |
| Address of depositary institution (including postal code and country) * | |
| 12301 Parklawn Drive Rockville, MD 20852 US | |
| Date of deposit * <u>September 14, 1994</u> Accession Number * <u>75885</u> | |
| B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet | |
| | |
| C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not all designated States) | |
| | |
| D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable) | |
| The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit") | |
| | |
| E. <input type="checkbox"/> This sheet was received with the International application when filed (to be checked by the receiving Office) | |
| | |
| (Authorized Officer) | |
| <input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau * | |
| was | |
| (Authorized Officer) | |

Form PCT/RO/134 (January 1981)

International Application No: PCT/

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

| <u>Accession No.</u> | <u>Date of Deposit</u> |
|----------------------|------------------------|
| 75886 | September 14, 1994 |
| 75887 | September 14, 1994 |
| 75888 | September 14, 1994 |
| 75889 | September 14, 1994 |
| 75890 | September 14, 1994 |
| 75970 | December 14, 1994 |
| 75971 | December 14, 1994 |
| 75972 | December 14, 1994 |
| 75973 | December 14, 1994 |
| 75974 | December 14, 1994 |

WHAT IS CLAIMED IS:

1. A method for detecting a defective OB-R in cells comprising:
 - (a) extracting RNA from a cell population;
 - 5 (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 1 in Figure 2; and
 - (c) detecting hybridization of the RNA with the oligonucleotide.
- 10 2. The method of Claim 1 in which the cell population is obtained from ovary.
3. The method of Claim 1 in which the cell population
15 is obtained from prostate.
4. The method of Claim 1 in which the cell population is obtained from testis.
- 20 5. The method of Claim 1 in which the cell population is obtained from sperm.
6. The method of Claim 1 in which the cell population is obtained from ovum.
- 25 7. The method of Claim 1 in which the cell population is obtained from cells of ovarian follicular fluids.
8. The method of Claim 1 in which the cell population
30 is obtained from blood.
9. A method for detecting a defective OB-R in cells comprising:
 - (a) extracting RNA from a cell population;
 - 35 (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 2 in Figure 2; and

(c) detecting hybridization of the RNA with the oligonucleotide.

10. The method of Claim 9 in which the cell population 5 is obtained from ovary.

11. The method of Claim 9 in which the cell population is obtained from prostate.

10 12. The method of Claim 9 in which the cell population is obtained from testis.

13. The method of Claim 9 in which the cell population is obtained from sperm.

15

14. The method of Claim 9 in which the cell population is obtained from ovum.

15. The method of Claim 9 in which the cell population 20 is obtained from cells of ovarian follicular fluids.

16. The method of Claim 9 in which the cell population is obtained from blood.

25 17. A method for detecting a defective OB-R in cells comprising:

- 30 (a) extracting RNA from a cell population;
(b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 3 in Figure 2; and
(c) detecting hybridization of the RNA with the oligonucleotide.

18. The method of Claim 17 in which the cell population 35 is obtained from ovary.

19. The method of Claim 17 in which the cell population is obtained from prostate.

20. The method of Claim 17 in which the cell population is obtained from testis.

21. The method of Claim 17 in which the cell population is obtained from sperm.

10 22. The method of Claim 17 in which the cell population is obtained from ovum.

23. The method of Claim 17 in which the cell population is obtained from cells of ovarian follicular fluids.

15

24. The method of Claim 17 in which the cell population is obtained from blood.

25. A method for treating infertility, comprising
20 administering to an individual an effective amount of an agent capable of inhibiting expression of an OB-R variant gene.

26. The method of Claim 25 in which the OB-R variant gene further comprises the sequence of Figure 2 or which is
25 capable of selectively hybridizing to it.

27. The method of Claim 26 in which the agent is an antisense molecule complementary to mRNA encoded by the sequence of Figure 2.

30

28. The method of Claim 26 in which the agent is a ribozyme molecule specific for mRNA encoded by the sequence of Figure 2.

35 29. The method of Claim 26 in which the agent is a triple helix component.

30. A method for identifying a compound capable of supplementing biological activity of leptin on cells of reproductive organs, comprising:

- 5 (a) incubating the cells expressing an OB-R variant with leptin;
- (b) incubating a portion of the leptin-treated cells with a test compound; and
- 10 (c) comparing activation signal in the cells treated in step (b) with cells treated in step (a);
- thereby determining the compound that augments activation of the OB-R variant by leptin.

31. The method of Claim 30 in which the OB-R variant 15 is encoded by the sequence depicted in Figure 2.

32. A method for identifying a compound capable of inhibiting biological activity of leptin on cells of reproductive organs, comprising:

- 20 (a) incubating the cells expressing an OB-R variant with leptin;
- (b) incubating a portion of the leptin-treated cells with a test compound; and
- 25 (c) comparing activation signal in the cells treated in step (b) with cells treated in step (a);
- thereby determining the compound that inhibits activation of the OB-R variant by leptin.

30 33. The method of Claim 32 in which the OB-R variant is encoded by the sequence depicted in Figure 2.

9 13 27 36 45 54
GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC TTC GGT
A R A T Q V P E P R P A P I S A F G

63 72 81 90 99 108
CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG ATG ATT TGT CAA
R V G P P D Q G V L L * S K M I C Q

117 126 135 144 153 162
AAA TTC TGT GTG GTT TTA CAT TGG GAA TTT ATT TAT GTG ATA ACT GCG TTT
K F C V V L L H W E F I Y V I T A F

171 180 189 198 207 216
AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA TTT AAG TTG TCT TGC ATG CCA CCA
N L S Y P I T P W R F K L S C M P P

225 234 243 252 261 270
AAT TCA ACC TAT GAC TAC TTC CTT TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA
N S T Y D Y F L L P A G L S K N T S

279 288 297 306 315 324
AAT TCG AAT GGA CAT TAT GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT
N S N G H Y E T A V E P K F N S S G

333 342 351 360 369 378
ACT CAC TTT TCT AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CCG AGT GAG
T H F S N L S K A T F H C C F R S E

387 396 405 414 423 432
CAA GAT AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT
Q D R N C S L C A D N I E G R T F V

441 450 459 468 477 486
TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC ATA CAG
S T V N S L V F Q Q I D A N W N I Q

495 504 513 522 531 540
TGC TGG CTA AAA GGA GAC TTA AAA TTA TTC ATC TGT TAT GTG GAG TCA TTA TTT
C W L K G D L K L F I C Y V E S L F

549 558 567 576 585 594
AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT CTT TTA TAT GTT CTG CCT
K N L F R N Y N Y K V H L L Y V L P

603 612 621 630 639 648
GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC CAA AAA GGC AGT TTT CAG ATG GTT
E V L E D S P L V P Q K G S F Q M V

Figure 1A

2/11

| | | | | | | | | |
|-----|-----|------|-----|------|------|------|------|------|
| CAC | TGC | 657 | TGC | 666 | 675 | 684 | 693 | 702 |
| H | C | N | C | S | V | H | E | C |
| GCC | AAA | 711 | AAC | 720 | 729 | 738 | 747 | 756 |
| A | K | L | N | D | T | L | M | C |
| ATT | TTC | 765 | TCA | 774 | 783 | 792 | 801 | 810 |
| I | F | R | S | P | L | M | S | V |
| CCA | CCA | 819 | TTA | 828 | 837 | 846 | 855 | 864 |
| P | P | L | G | L | H | M | E | I |
| TGG | TCC | 873 | CCA | 882 | 891 | 900 | 909 | 918 |
| W | S | S | P | P | L | V | P | F |
| GAG | AAT | 927 | ACA | 936 | 945 | 954 | 963 | 972 |
| E | N | S | T | T | V | I | R | E |
| CTG | CTA | 981 | GAC | 990 | 999 | 1008 | 1017 | 1026 |
| L | L | V | D | S | I | L | P | G |
| AAG | AGA | 1035 | GAT | 1044 | 1053 | 1062 | 1071 | 1080 |
| K | R | L | D | G | P | G | I | W |
| ACC | ACA | 1089 | GAT | 1098 | 1107 | 1116 | 1125 | 1134 |
| T | T | Q | D | V | I | Y | F | P |
| AAT | GTT | 1143 | TTT | 1152 | 1161 | 1170 | 1179 | 1188 |
| N | V | S | F | H | C | I | Y | K |
| GAG | ATT | 1197 | TGG | 1206 | 1215 | 1224 | 1233 | 1242 |
| E | I | V | W | W | M | N | L | A |
| GTT | GTG | 1251 | GAT | 1260 | 1269 | 1278 | 1287 | 1296 |
| V | V | S | D | H | V | S | K | V |
| CCT | CGA | 1305 | AAG | 1314 | 1323 | 1332 | 1341 | 1350 |
| P | R | G | K | F | T | Y | D | A |

Figure 1B

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CAT | CAT | CGC | TAT | GCT | GAA | TTA | TAT | GTG | ATT | GAT | GTC | AAT | ATC | AAT | ATC | TCA | TGT |
| H | H | R | Y | A | E | L | Y | V | I | D | V | N | I | N | I | S | C |
| GAA | ACT | GAT | GGG | TAC | TTA | ACT | AAA | ATG | ACT | TGC | AGA | TGG | TCA | ACC | AGT | ACA | ATC |
| E | T | D | G | Y | L | T | K | M | T | C | R | W | S | T | S | T | I |
| CAG | TCA | CTT | GCG | GAA | AGC | ACT | TTG | CAA | TTG | AGG | TAT | CAT | AGG | AGC | AGC | CTT | TAC |
| Q | S | L | A | E | S | T | L | Q | L | R | Y | H | R | S | S | L | Y |
| TGT | TCT | GAT | ATT | CCA | TCT | ATT | CAT | CCC | ATA | TCT | GAG | CCC | AAA | GAT | TGC | TAT | TTG |
| C | S | D | I | P | S | I | H | P | I | S | E | P | K | D | C | Y | L |
| CAG | AGT | GAT | GGT | TTT | TAT | GAA | TGC | ATT | TTC | CAG | CCA | ATC | TTC | CTA | TTA | TCT | GGC |
| Q | S | D | G | F | Y | E | C | I | F | Q | P | I | F | L | L | S | G |
| TAC | ACA | ATG | TGG | ATT | AGG | ATC | AAT | CAC | TCT | CTA | GGT | TCA | CTT | GAC | TCT | CCA | CCA |
| Y | T | M | W | I | R | I | N | H | S | L | G | S | L | D | S | P | P |
| ACA | TGT | GTC | CTT | CCT | GAT | TCT | GTG | GTG | AAG | CCA | CTG | CCT | CCA | TCC | AGT | GTG | AAA |
| T | C | V | L | P | D | S | V | V | K | P | L | P | P | S | S | V | K |
| GCA | GAA | ATT | ACT | ATA | AAC | ATT | GGA | TTA | TTG | AAA | ATA | TCT | TGG | GAA | AAG | CCA | GTC |
| A | E | I | T | I | N | I | G | L | L | K | I | S | W | E | K | P | V |
| TTT | CCA | GAG | AAT | AAC | CTT | CAA | TTC | CAG | ATT | CGC | TAT | GGT | TTA | AGT | GGA | AAA | GAA |
| F | P | E | N | N | L | Q | F | Q | I | R | Y | G | L | S | G | K | E |
| GTA | CAA | TGG | AAG | ATG | TAT | GAG | GTT | TAT | GAT | GCA | AAA | TCA | AAA | TCT | GTC | AGT | CTC |
| V | Q | W | K | M | Y | E | V | Y | D | A | K | S | K | S | V | S | L |
| CCA | GTT | CCA | GAC | TTG | TGT | GCA | GTC | TAT | GCT | GTT | CAG | GTG | CGC | TGT | AAG | AGG | CTA |
| P | V | P | D | L | C | A | V | Y | A | V | Q | V | R | C | K | R | L |
| GAT | GGA | CTG | GGA | TAT | TGG | AGT | AAT | TGG | AGC | AAT | CCA | GCC | TAC | ACA | GTT | GTC | ATG |
| D | G | L | G | Y | W | S | N | W | S | N | P | A | Y | T | V | V | H |
| GAT | ATA | AAA | GTT | CCT | ATG | AGA | GGA | CCT | GAA | TTT | TGG | AGA | ATA | ATT | AAT | GGA | GAT |
| D | I | K | V | P | M | R | G | P | E | F | W | R | I | I | N | G | D |

Figure 1C

| | | | | | |
|-----------------|-----------------|-----------------|-----------------|-------------|------|
| 2061 | 2070 | 2079 | 2088 | 2097 | 2106 |
| ACT ATG AAA AAG | GAG AAA AAT GTC | ACT TTA CTT TGG | AAG CCC CTG | ATG AAA AAT | |
| T M K K | E K N V | T L L W | K P L | M K N | |
| 2115 | 2124 | 2133 | 2142 | 2151 | 2160 |
| GAC TCA TTG TGC | AGT GTT CAG AGA | TAT GTG ATA AAC | CAT CAT ACT | TOC TGC AAT | |
| D S L C | S V Q R | Y V I N | H H T | S C N | |
| 2169 | 2178 | 2187 | 2196 | 2205 | 2214 |
| GGA ACA TGG TCA | GAA GAT GTG GGA | AAT CAC ACG AAA | TTC ACT TTC | CTG TGG ACA | |
| G T W S | E D V G | N H T K | F T F | L W T | |
| 2223 | 2232 | 2241 | 2250 | 2259 | 2268 |
| GAG CAA GCA CAT | ACT GTT ACG GTT | CTG GCC ATC AAT | TCA ATT GGT | GCT TCT GTT | |
| E Q A H | T V T V | L A I N | S I G | A S V | |
| 2277 | 2286 | 2295 | 2304 | 2313 | 2322 |
| GCA AAT TTT AAT | TTA ACC TTT TCA | TGG OCT ATG AGC | AAA GTA AAT | ATC GTG CAG | |
| A N F N | L T F S | W P H S | K V N | I V Q | |
| 2331 | 2340 | 2349 | 2358 | 2367 | 2376 |
| TCA CTC AGT GCT | TAT CCT TTA AAC | AGC AGT TGT GTG | ATT GTT TCC | TGG ATA CTA | |
| S L S A | Y P L N | S S C V | I V S | W I L | |
| 2385 | 2394 | 2403 | 2412 | 2421 | 2430 |
| TCA CCC AGT GAT | TAC AAG CTA ATG | TAT TTT ATT ATT | GAG TGG AAA | AAT CTT AAT | |
| S P S D | Y K L M | Y F I I | E W K N | L N | |
| 2439 | 2448 | 2457 | 2466 | 2475 | 2484 |
| GAA GAT GGT GAA | ATA AAA TGG CTT | AGA ATC TCT TCA | TCT GTT AAG | AAG TAT TAT | |
| E D G E | I K W L | R I S S | S V K K | Y Y | |
| 2493 | 2502 | 2511 | 2520 | 2529 | 2538 |
| ATC CAT GAT CAT | TTT ATC CCC ATT | GAG AAG TAC CAG | TTC AGT CTT | TAC CCA ATA | |
| I H D H | F I P I | E K Y Q | F S L Y | P I | |
| 2547 | 2556 | 2565 | 2574 | 2583 | 2592 |
| TTT ATG GAA GGA | GTG GGA AAA CCA | AAG ATA ATT AAT | AGT TTC ACT | CAA GAT GAT | |
| F M E G | V G K P | K I I N | S F T Q | D D | |
| 2601 | 2610 | 2619 | 2628 | 2637 | 2646 |
| ATT GAA AAA CAC | CAG AGT GAT GCA | GGT TTA TAT GTA | ATT GTG CCA | GTA ATT ATT | |
| I E K H | Q S D A | G L Y V | I V P V | I I | |
| 2655 | 2664 | 2673 | 2682 | 2691 | 2700 |
| TCC TCT TCC ATC | TTA TTG CTT GGA | ACA TTA TTA ATA | TCA CAC CAA | AGA ATG AAA | |
| S S S I | L L L G | T L L I | S H Q R | M K | |
| 2709 | 2718 | 2727 | 2736 | 2745 | 2754 |
| AAG CTA TTT TGG | GAA GAT GTT CCG | AAC CCC AAG | AAT TGT TCC TGG | GCA CAA GGA | |
| K L F W | E D V P | N P K N | C S W A | Q G | |

Figure 1D

| | | | | | | | | | | | | | | | | | |
|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|
| 2763 | | | 2772 | | | 2781 | | | 2790 | | | 2799 | | | 2808 | | |
| CTT | AAT | TTT | CAG | AAG | ATG | CTT | GAA | GGC | AGC | ATG | TTC | GTT | AAG | AGT | CAT | CAC | CAC |
| L | N | F | Q | K | M | L | E | G | S | M | F | V | K | S | H | H | H |
| 2817 | | | 2826 | | | 2835 | | | 2844 | | | 2853 | | | 2862 | | |
| TCC | CTA | ATC | TCA | AGT | ACC | CAG | GGA | CAC | AAA | CAC | TGC | GGA | AGG | CCA | CAG | GGT | CCT |
| S | L | I | S | S | T | Q | G | H | K | H | C | G | R | P | Q | G | P |
| 2871 | | | 2880 | | | 2889 | | | 2898 | | | 2907 | | | 2916 | | |
| CTG | CAT | AGG | AAA | ACC | AGA | GAC | CTT | TGT | TCA | CTT | GTT | TAT | CTG | CTG | ACC | CTC | CCT |
| L | H | R | K | T | R | D | L | C | S | L | V | Y | L | L | T | L | P |
| 2925 | | | 2934 | | | 2943 | | | 2952 | | | 2961 | | | 2970 | | |
| CCA | CTA | TTG | TCC | TAT | GAC | CCT | GCC | AAA | TCC | CCC | TCT | GTG | AGA | AAC | ACC | CAA | GAA |
| P | L | L | S | Y | D | P | A | K | S | P | S | V | R | N | T | Q | E |
| 2979 | | | 2988 | | | | | | | | | | | | | | |
| TGA | TCA | ATA | AAA | AAA | AAA | AAA | 3' | | | | | | | | | | |
| * | S | I | K | K | K | K | | | | | | | | | | | |

Figure 1 E

| | | | | | | | | |
|------|---|------|------------|------------|------------|------------|------------|------|
| Form | 1 | 2751 | 2760 | 2770 | 2780 | 2790 | 2800 | |
| | 2 | 2751 | AGGACTTAAT | TTTCAGAAGA | TCCTTGAAGG | CAGCATGTTT | GTTAAGAGTC | 2800 |
| | 3 | 2751 | AGGACTTAAT | TTTCAGAAGA | AAATGCCTGG | CACAAAGGAA | CTACTGGGTG | 2800 |
| Form | 1 | 2751 | AGGACTTAAT | TTTCAGAAGA | GAACGGACAT | TCCTTGAAGT | CTAATCATEA | 2800 |
| | 2 | 2801 | 2810 | 2820 | 2830 | 2840 | 2850 | |
| | 3 | 2801 | ATCACCACTC | CCTAATCTCA | AGTACCCAGG | GACACAAACA | CTGCGGAAGG | 2850 |
| Form | 1 | 2801 | GAGGTGGTT | GACTTAGGAA | ATGCTTGTA | AGCTACGTCC | TACCTGGTGC | 2850 |
| | 2 | 2801 | TCACACAGA | TGAACCCAAT | GTGCCAACTT | CCCAACAGTC | TATAGAGTAT | 2850 |
| | 3 | 2801 | TCACACAGA | TGAACCCAAT | GTGCCAACTT | CCCAACAGTC | TATAGAGTAT | 2850 |
| Form | 1 | 2851 | 2860 | 2870 | 2880 | 2890 | 2900 | |
| | 2 | 2851 | CCACAGGGTC | CTCTGCATAG | GAAAACCAGA | GACCTTTGTT | CACTTGTTTA | 2900 |
| | 3 | 2851 | GCACCTGCTC | TCCCTGAGGT | GTGCACAATG | | | 2900 |
| Form | 1 | 2851 | TAGAAGATT | TTACATTCTG | AAGAAGG... | | | 2900 |
| | 2 | 2901 | 2910 | 2920 | 2930 | 2940 | 2950 | |
| | 3 | 2901 | TCTGCTGACC | CTCCCTOCAC | TATTGTCCTA | TGACCCTGCC | AAATCCCOCT | 2950 |
| Form | 1 | 2901 | | | | | | 2950 |
| | 2 | 2901 | | | | | | 2950 |
| | 3 | 2901 | | | | | | 2950 |
| Form | 1 | 2951 | 2960 | 2970 | 2980 | 2990 | 3000 | |
| | 2 | 2951 | CTGTGAGAAA | CACCCAAGAA | TGATCAATAA | AAAAAAAAA | A..... | 3000 |
| | 3 | 2951 | | | | | | 3000 |
| Form | 1 | 2951 | | | | | | 3000 |
| | 2 | 2951 | | | | | | 3000 |
| | 3 | 2951 | | | | | | 3000 |

Figure 2.

| | | | | | | | |
|------------|-----|------------|------------|------------|------------|------------|-----|
| | | 10 | 20 | 30 | 40 | 50 | |
| HuB1.219_1 | 1 | MIGGKGVAVL | LHWELLYVAT | AFNLSYPLTF | WRFKLSGMP | NSTVDYVLLP | |
| HuB1.219_2 | 1 | MIGGKGVAVL | LHWELLYVAT | AFNLSYPLTF | WRFKLSGMP | NSTVDYVLLP | 50 |
| HuB1.219_3 | 1 | MIGGKGVAVL | LHWELLYVAT | AFNLSYPLTF | WRFKLSGMP | NSTVDYVLLP | 50 |
| HuOBR | 1 | MIGGKGVAVL | LHWELLYVAT | AFNLSYPLTF | WRFKLSGMP | NSTVDYVLLP | 50 |
| MuOBR | 1 | MIGGKGVAVL | LHWELLYVAT | AFNLSYPLTF | WRFKLSGMP | NSTVDYVLLP | 50 |
| | | 60 | 70 | 80 | 90 | 100 | |
| HuB1.219_1 | 51 | AGLSKNTSNE | NCHYTAVVE | KFNSSGTHFS | NESKATFHCC | FRSPDQDNCS | |
| HuB1.219_2 | 51 | AGLSKNTSNE | NCHYTAVVE | KFNSSGTHFS | NESKATFHCC | FRSPDQDNCS | 100 |
| HuB1.219_3 | 51 | AGLSKNTSNE | NCHYTAVVE | KFNSSGTHFS | NESKATFHCC | FRSPDQDNCS | 100 |
| HuOBR | 51 | AGLSKNTSNE | NCHYTAVVE | KFNSSGTHFS | NESKATFHCC | FRSPDQDNCS | 100 |
| MuOBR | 51 | AGAPNNAAL | KGASEATVA | KFNSSGTHVP | ESKIVFHCC | FGNDOQDNCS | 100 |
| | | 110 | 120 | 130 | 140 | 150 | |
| HuB1.219_1 | 101 | QZRNDEGRT | RSTUNSLVE | QSIDANNTIC | QWLKGDDEKE | ICVYESLFKN | |
| HuB1.219_2 | 101 | QZRNDEGRT | RSTUNSLVE | QSIDANNTIC | QWLKGDDEKE | ICVYESLFKN | 150 |
| HuB1.219_3 | 101 | QZRNDEGRT | RSTUNSLVE | QSIDANNTIC | QWLKGDDEKE | ICVYESLFKN | 150 |
| HuOBR | 101 | QZRNDEGRT | RSTUNSLVE | QSIDANNTIC | QWLKGDDEKE | ICVYESLFKN | 150 |
| MuOBR | 101 | ALTETETET | LASVVKASVE | RGLGVNDIE | QWLKGDDEKE | ICVYESLFKN | 150 |
| | | 160 | 170 | 180 | 190 | 200 | |
| HuB1.219_1 | 151 | LENNYNYKVM | LYSLDPEVLE | DSPLVFGKGS | FOVYHNCESV | HCCEGLVAV | |
| HuB1.219_2 | 151 | LENNYNYKVM | LYSLDPEVLE | DSPLVFGKGS | FOVYHNCESV | HCCEGLVAV | 200 |
| HuB1.219_3 | 151 | LENNYNYKVM | LYSLDPEVLE | DSPLVFGKGS | FOVYHNCESV | HCCEGLVAV | 200 |
| HuOBR | 151 | LENNYNYKVM | LYSLDPEVLE | DSPLVFGKGS | FOVYHNCESV | HCCEGLVAV | 200 |
| MuOBR | 151 | PERKEDS | LHDEEDD | DSPEFLKDS | ROTQCNEL | RGC-EDVVPV | 200 |
| | | 210 | 220 | 230 | 240 | 250 | |
| HuB1.219_1 | 201 | ETALNDRTH | HGLKATSGCA | EFSELMSSVO | PIMVVKEDPP | LGLHMETTDD | |
| HuB1.219_2 | 201 | ETALNDRTH | HGLKATSGCA | EFSELMSSVO | PIMVVKEDPP | LGLHMETTDD | 250 |
| HuB1.219_3 | 201 | ETALNDRTH | HGLKATSGCA | EFSELMSSVO | PIMVVKEDPP | LGLHMETTDD | 250 |
| HuOBR | 201 | ETALNDRTH | HGLKATSGCA | EFSELMSSVO | PIMVVKEDPP | LGLHMETTDD | 250 |
| MuOBR | 201 | FRKGVAAE | MLEETGAGV | SROSLMSLO | PMLVVKEDPP | LGLHMETTDD | 250 |
| | | 260 | 270 | 280 | 290 | 300 | |
| HuB1.219_1 | 251 | GNLQTSNSSF | PIVPPRFXQO | VRYSENSTTV | IREADKIVSA | TSLLVDSILP | |
| HuB1.219_2 | 251 | GNLQTSNSSF | PIVPPRFXQO | VRYSENSTTV | IREADKIVSA | TSLLVDSILP | 300 |
| HuB1.219_3 | 251 | GNLQTSNSSF | PIVPPRFXQO | VRYSENSTTV | IREADKIVSA | TSLLVDSILP | 300 |
| HuOBR | 251 | GNLQTSNSSF | PIVPPRFXQO | VRYSENSTTV | IREADKIVSA | TSLLVDSILP | 300 |
| MuOBR | 251 | GNLQTSNSSF | PIVPPRFXQO | VRYSENSTTV | IREADKIVSA | TSLLVDSILP | 300 |
| | | 310 | 320 | 330 | 340 | 350 | |
| HuB1.219_1 | 301 | GSSYGVVWRS | KHLDCGGLWS | QWSTPRVETL | QDVTYFPKPI | LTSVGSNVSP | |
| HuB1.219_2 | 301 | GSSYGVVWRS | KHLDCGGLWS | QWSTPRVETL | QDVTYFPKPI | LTSVGSNVSP | 350 |
| HuB1.219_3 | 301 | GSSYGVVWRS | KHLDCGGLWS | QWSTPRVETL | QDVTYFPKPI | LTSVGSNVSP | 350 |
| HuOBR | 301 | GSSYGVVWRS | KHLDCGGLWS | QWSTPRVETL | QDVTYFPKPI | LTSVGSNVSP | 350 |
| MuOBR | 301 | GSSYGVVWRS | KHLDCGGLWS | QWSTPRVETL | QDVTYFPKPI | LTSVGSNVSP | 350 |
| | | 360 | 370 | 380 | 390 | 400 | |
| HuB1.219_1 | 351 | HGLYKKNVCE | VPSKPTUWAM | NLAELKPGSO | VDAVSDRVSK | VTFPNLNETH | |
| HuB1.219_2 | 351 | HGLYKKNVCE | VPSKPTUWAM | NLAELKPGSO | VDAVSDRVSK | VTFPNLNETH | 400 |
| HuB1.219_3 | 351 | HGLYKKNVCE | VPSKPTUWAM | NLAELKPGSO | VDAVSDRVSK | VTFPNLNETH | 400 |
| HuOBR | 351 | HGLYKKNVCE | VPSKPTUWAM | NLAELKPGSO | VDAVSDRVSK | VTFPNLNETH | 400 |
| MuOBR | 351 | HGLYKKNVCE | VPSKPTUWAM | NLAELKPGSO | VDAVSDRVSK | VTFPNLNETH | 400 |
| | | 410 | 420 | 430 | 440 | 450 | |
| HuB1.219_1 | 401 | PRGKETIDAV | YCCNEHCHH | RYAELVVIDV | NINISCETDG | YLTQTICRWS | |
| HuB1.219_2 | 401 | PRGKETIDAV | YCCNEHCHH | RYAELVVIDV | NINISCETDG | YLTQTICRWS | 450 |
| HuB1.219_3 | 401 | PRGKETIDAV | YCCNEHCHH | RYAELVVIDV | NINISCETDG | YLTQTICRWS | 450 |
| HuOBR | 401 | PRGKETIDAV | YCCNEHCHH | RYAELVVIDV | NINISCETDG | YLTQTICRWS | 450 |
| MuOBR | 401 | PRGKETIDAV | YCCNEHCHH | RYAELVVIDV | NINISCETDG | YLTQTICRWS | 450 |

Figure 3A

| | | | | | | | |
|------------|-----|------------|------------|-------------|------------|------------|-----|
| | | 460 | 470 | 480 | 490 | 500 | |
| HuB1.219_1 | 451 | TSTTOSTAES | TGOLRYHRSR | LYGSDIPISTH | PISEPKDGYT | QSDGFYECIB | 500 |
| HuB1.219_2 | 451 | TSTTOSTAES | TGOLRYHRSR | LYGSDIPISTH | PISEPKDGYT | QSDGFYECIB | 500 |
| HuB1.219_3 | 451 | TSTTOSTAES | TGOLRYHRSR | LYGSDIPISTH | PISEPKDGYT | QSDGFYECIB | 500 |
| HuOBR | 451 | TSTTOSTAES | TGOLRYHRSR | LYGSDIPISTH | PISEPKDGYT | QSDGFYECIB | 500 |
| MuOBR | 451 | PTTOSTAES | TGOLRYHRSR | LYGSDIPISTH | PISEPKDGYT | QSDGFYECIB | 500 |
| | | 510 | 520 | 530 | 540 | 550 | |
| HuB1.219_1 | 501 | QPTPLKSGY | MYRPNHSG | SLTSEPTGV | EDSVKPEPP | SSVAKETIN | 550 |
| HuB1.219_2 | 501 | QPTPLKSGY | MYRPNHSG | SLTSEPTGV | EDSVKPEPP | SSVAKETIN | 550 |
| HuB1.219_3 | 501 | QPTPLKSGY | MYRPNHSG | SLTSEPTGV | EDSVKPEPP | SSVAKETIN | 550 |
| HuOBR | 501 | QPTPLKSGY | MYRPNHSG | SLTSEPTGV | EDSVKPEPP | SSVAKETIN | 550 |
| MuOBR | 501 | QPTPLKSGY | MYRPNHSG | SLTSEPTGV | EDSVKPEPP | SSVAKETIN | 550 |
| | | 560 | 570 | 580 | 590 | 600 | |
| HuB1.219_1 | 551 | IGMLKSGY | MYRPNHSG | QIRYGLSGKE | VQWKLKVEAD | AKSKVSLV | 600 |
| HuB1.219_2 | 551 | IGMLKSGY | MYRPNHSG | QIRYGLSGKE | VQWKLKVEAD | AKSKVSLV | 600 |
| HuB1.219_3 | 551 | IGMLKSGY | MYRPNHSG | QIRYGLSGKE | VQWKLKVEAD | AKSKVSLV | 600 |
| HuOBR | 551 | IGMLKSGY | MYRPNHSG | QIRYGLSGKE | VQWKLKVEAD | AKSKVSLV | 600 |
| MuOBR | 551 | TGMLKSGY | MYRPNHSG | QIRYGLSGKE | VQWKLKVEAD | AKSKVSLV | 600 |
| | | 610 | 620 | 630 | 640 | 650 | |
| HuB1.219_1 | 601 | PDECAVAVG | VRCRDPGIG | VSNWSNPAY | LVMDIKVPA | RGPEFWKIN | 650 |
| HuB1.219_2 | 601 | PDECAVAVG | VRCRDPGIG | VSNWSNPAY | LVMDIKVPA | RGPEFWKIN | 650 |
| HuB1.219_3 | 601 | PDECAVAVG | VRCRDPGIG | VSNWSNPAY | LVMDIKVPA | RGPEFWKIN | 650 |
| HuOBR | 601 | PDECAVAVG | VRCRDPGIG | VSNWSNPAY | LVMDIKVPA | RGPEFWKIN | 650 |
| MuOBR | 601 | SPICAVAVG | VRCRDPGIG | VSNWSNPAY | LVMDIKVPA | RGPEFWKIN | 650 |
| | | 660 | 670 | 680 | 690 | 700 | |
| HuB1.219_1 | 651 | GDVTKRNV | TLTKELTN | DSLCVQRV | INHTSENG | WSEVGNK | 700 |
| HuB1.219_2 | 651 | GDVTKRNV | TLTKELTN | DSLCVQRV | INHTSENG | WSEVGNK | 700 |
| HuB1.219_3 | 651 | GDVTKRNV | TLTKELTN | DSLCVQRV | INHTSENG | WSEVGNK | 700 |
| HuOBR | 651 | GDVTKRNV | TLTKELTN | DSLCVQRV | INHTSENG | WSEVGNK | 700 |
| MuOBR | 651 | GDVTKRNV | TLTKELTN | DSLCVQRV | INHTSENG | WSEVGNK | 700 |
| | | 710 | 720 | 730 | 740 | 750 | |
| HuB1.219_1 | 701 | ETEDVTEAR | TVNANST | GASVANFNT | ESWPSKVN | VQSLSAVFN | 750 |
| HuB1.219_2 | 701 | ETEDVTEAR | TVNANST | GASVANFNT | ESWPSKVN | VQSLSAVFN | 750 |
| HuB1.219_3 | 701 | ETEDVTEAR | TVNANST | GASVANFNT | ESWPSKVN | VQSLSAVFN | 750 |
| HuOBR | 701 | ETEDVTEAR | TVNANST | GASVANFNT | ESWPSKVN | VQSLSAVFN | 750 |
| MuOBR | 701 | ETEDVTEAR | TVNANST | GASVANFNT | ESWPSKVN | VQSLSAVFN | 750 |
| | | 760 | 770 | 780 | 790 | 800 | |
| HuB1.219_1 | 751 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 800 |
| HuB1.219_2 | 751 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 800 |
| HuB1.219_3 | 751 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 800 |
| HuOBR | 751 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 800 |
| MuOBR | 751 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 800 |
| | | 810 | 820 | 830 | 840 | 850 | |
| HuB1.219_1 | 801 | ETEDVTEAR | TVNANST | KPKINSETO | DDIEKHOSDA | GLYVIVPVI | 850 |
| HuB1.219_2 | 801 | ETEDVTEAR | TVNANST | KPKINSETO | DDIEKHOSDA | GLYVIVPVI | 850 |
| HuB1.219_3 | 801 | ETEDVTEAR | TVNANST | KPKINSETO | DDIEKHOSDA | GLYVIVPVI | 850 |
| HuOBR | 801 | ETEDVTEAR | TVNANST | KPKINSETO | DDIEKHOSDA | GLYVIVPVI | 850 |
| MuOBR | 801 | ETEDVTEAR | TVNANST | KPKINSETO | DDIEKHOSDA | GLYVIVPVI | 850 |
| | | 860 | 870 | 880 | 890 | 900 | |
| HuB1.219_1 | 851 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 900 |
| HuB1.219_2 | 851 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 900 |
| HuB1.219_3 | 851 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 900 |
| HuOBR | 851 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 900 |
| MuOBR | 851 | SSQVAVN | SPSDGQHYE | YEWKLNED | GETRWLRSS | SVKQYTHD | 900 |

Figure 3 B

| | | | | | | | |
|------------|------|------------|------------|------------|------------|------------|------|
| Hub1.219_1 | 901 | 910 | 920 | 930 | 940 | 950 | |
| Hub1.219_2 | 901 | SHHSLISST | QGHKHCGRPQ | GPLHRKTRDL | CSLVYLLALP | PHSYDPAKS | 950 |
| Hub1.219_3 | 901 | GGGWL..... | | | | | 950 |
| HuOBR | 901 | | | | | | 950 |
| MuOBR | 901 | KHTASVTGTF | LLEPETISE | DISVDTSWKN | KDEMPTEVV | SEESTIDLEK | 950 |
| | 901 | | | | | | 950 |
| Hub1.219_1 | 951 | 960 | 970 | 980 | 990 | 1000 | |
| Hub1.219_2 | 951 | PSYRATOE.. | | | | | 1000 |
| Hub1.219_3 | 951 | | | | | | 1000 |
| HuOBR | 951 | GMICISDFN | SVNFSEAGT | EVTYEAESQ | QPFVKYATLI | SNSKPSETGE | 1000 |
| MuOBR | 951 | | | | | | 1000 |
| Hub1.219_1 | 1001 | 1010 | 1020 | 1030 | 1040 | 1050 | |
| Hub1.219_2 | 1001 | | | | | | 1050 |
| Hub1.219_3 | 1001 | | | | | | 1050 |
| HuOBR | 1001 | EQGLINSSVT | KCFSSKNSPL | KDSFSNSSWE | TEAQAFFILS | DQHPNLIISH | 1050 |
| MuOBR | 1001 | | | | | | 1050 |
| Hub1.219_1 | 1051 | 1060 | 1070 | 1080 | 1090 | 1100 | |
| Hub1.219_2 | 1051 | | | | | | 1100 |
| Hub1.219_3 | 1051 | | | | | | 1100 |
| HuOBR | 1051 | LTFSEGLDEL | LKLEGNFPEE | NNDKXSIYYL | GVTSIKKRES | GVLLTDKSRV | 1100 |
| MuOBR | 1051 | | | | | | 1100 |
| Hub1.219_1 | 1101 | 1110 | 1120 | 1130 | 1140 | 1150 | |
| Hub1.219_2 | 1101 | | | | | | 1150 |
| Hub1.219_3 | 1101 | | | | | | 1150 |
| HuOBR | 1101 | SCFFPAPCLF | TDIRVLQDSC | SHFVENNINL | GTSSKKTFAS | YMPQFQTCST | 1150 |
| MuOBR | 1101 | | | | | | 1150 |
| Hub1.219_1 | 1151 | 1160 | 1170 | 1180 | 1190 | 1200 | |
| Hub1.219_2 | 1151 | | | | | | 1200 |
| Hub1.219_3 | 1151 | | | | | | 1200 |
| HuOBR | 1151 | QTHKIMENKH | CDLTV* | | | | 1200 |
| MuOBR | 1151 | | | | | | 1200 |

Figure 3c

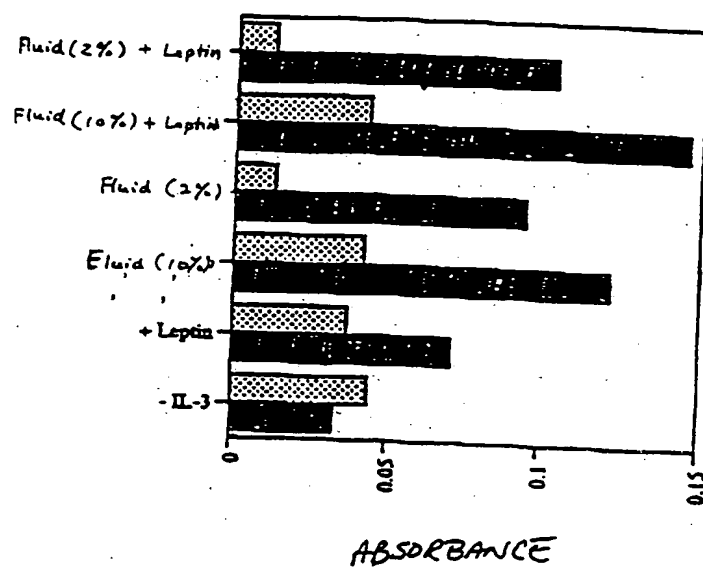


Figure 4

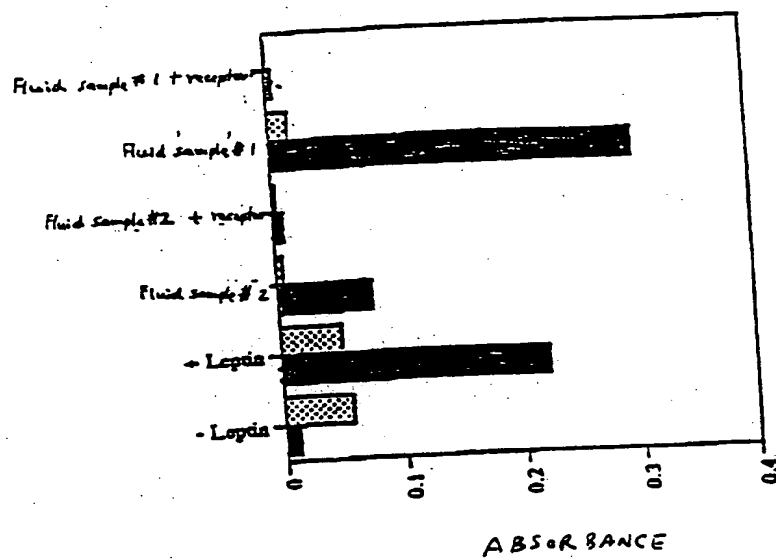


Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US97/07676

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12P 19/34; C07H 21/02, 21/04

US CL : 435/6, 91.2; 536/23.1, 24.3, 24.33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/23.1, 24.3, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|--------------|---|--|
| Y -- A | TARTAGLIA, L.A. et al., Identification and Expression Cloning of a Leptin Receptor, OB-R. Cell . 29 December 1995. Volume 83, pages 1263-1271, see entire document. | 1,9,17, 25,26,30, 32 ----- 2-8, 10-16, 18- 24, 27-29, 31, 33 |



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z

document member of the same patent family

Date of the actual completion of the international search

04 JULY 1997

Date of mailing of the international search report

04.08.97

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DIANNE REES

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/07676

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used).

APS, BIOSIS, BIOTECHABS, BIOTECHDS, CABA, CAPLUS, CANCERLIT, EMBASE, MEDLINE, USPATFULL, EUROPATFULL, JAPIO, GENBANK, SCISEARCH, LIFESCI, TOXLINE, TOXLIT, DRUGU

search terms: leptin receptor, OB-R, probes, primers, sequence, cloning, diagnosis, mutation detection, alleles, variants, forms, isoforms, ribozymes, antisense, triple helix, screening, drugs